THE INFLUENCE OF SEX HORMONES ON THE LEUCOCYTE COUNT OF THE CHICKEN

by

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INTRODUCTION

The blood cells of chickens have been studied for many years, and while most authors agree on the number of erythrocytes, they do not agree so well on the total leucocyte count.

Until recently no satisfactory method of staining was known as most of the stains used caused hemolysis of the nucleated red blood cells giving them the appearance of leucocytes. Before the year 1928, an indirect method of counting was used. This method gave varied and extremely large numbers of leucocytes per cubic millimeter. Further complications resulted from the counts of individual birds, which vary from day to day, or even from hour to hour. It also has been found that the male has a higher average count than does the female bird.

The purpose of this work was to determine if the noticeable differences in the total leucocyte count of male and female were due, at least in part, to the sex hormones. By using the male, female, and capon chickens, and by injecting these with sex hormone, it was hoped that the cause for the differences between the sexes would be found.

REVIEW OF LITERATURE

Literature on the total leucocyte count for chickens is abundant. The different totals given are nearly as varied as the investigators making the counts. Few of the authors gave

the age, sex, or time of making the count, all of which are important in establishing an average count for the chicken.

No reports on the effect of sex hormones on the leucocytes of the chicken could be found, although the effect of these hormones on the white blood cell count of other animals has been recorded.

Tyslowitz and Hartman (1941) found that injections of estrogen has no significant effect on the total leucocyte count of male or female Rhesus monkeys.

Crafts (1941) injected 10 mg of stilbestrol per day into the Rhesus monkey and obtained an increase of 4,000 white blood cells at the end of four days. Oral administrations of stilbestrol resulted in a reduction of 5,000 cells per cu mm.

Jacobson (1944) obtained a slight increase in the total cell count by injecting estrogen into white mice. He obtained a larger total increase by injections of sterile saline solutions, however.

Taber and Domm (1942), counting the erythrocytes of Brown Leghorn pullets, found an increase of up to 50 percent following injections of testosterone propionate over a period of 65 days. Capons showed an increase of 50.5 percent. The same authors (1943) injected four capons with 1 mg of testosterone propionate daily for 72 days, by the twentieth day the erythrocyte level had reached that of the normal male. Pullets implanted with testosterone pellets reached male level in 16 days.

Taber, Davis, and Domm (1943) confirmed the results given

above, and obtained a decrease in erythrocyte count in males by injections of estrogen.

Grollman et al. (1940), using both testosterone and diethylstilbestrol injections in normal white rats, records an elevation of blood pressure, but did not record the leucocyte count.

Stein and Jacobsen (1944) counting the leucocytes in hamsters found no differences in total number between normal and castrate individuals.

The presence or absence of a digestive leucocyte in birds has been studied by various authors.

Palmer and Biely (1935) studied the leucocyte count in chickens during a period of starvation and immediately following the termination of the starvation period. Their results point toward the presence of a digestive leucocyte.

Blakemore (1934) recorded a slight variation in the afternoon but not enough to show the presence of a digestive leucocyte. A large daily variation was found with each individual bird following its own specific cycle, which was repeated at approximately weekly intervals.

Shaw (1930) found a diurnal rhythm in the pigeon. The afternoon count was up to 143 percent higher than the morning count. This cycle was followed in pigeons being starved, showing that a digestive leucocyte was lacking.

The portion of the body from which the blood is withdrawn gives a different leucocyte count.

Quimby, Saxton, and Goff (1948), working with white albino rats, found a difference of 17,000 white blood cells per cu mm between blood taken from the peripherium and the heart.

Twisselman (1937) withdrew blood from the tip of the combs of chickens. The range from this area being from 14,000 to 59,000 with a high average of 38,000 white blood cells. This average being considerably higher than that given by most authors.

Vejlens (1938) states that the speed at which the blood flows through the blood vessels determines in part the leucocyte count in a certain area. The smaller capillaries slow the flow of blood thus showing more white blood cells in them than is found in other portions of the body. Therefore, the first blood withdrawn from a skin puncture, or from an area of many capillaries, has an excess number of cells due to the paracapillary adherence of the cells.

Das Gupta (1939) suggests the following reasons for increases in the white blood cell count:

1. redistribution of circulating leucocytes.

2. mobilization of the granulocytic reserve of the bone marrow.

3. increase maturation of granulocytes.

The increase caused by methods 1 and 2 are temporary, lasting only a few hours, while those formed under method number 3 may be recognized for days.

Many external factors cause a change in the total leucocyte

count. Such things as excitement, exercise, or slight injury will result in an increase in the cell count, as was shown by Das Gupta.

The diet can cause an increase or decrease in total number of cells as was demonstrated by Campbell et al. (1945).

To minimize the effects of diet and other factors the birds used in these experiments were fed the same diet and kept under as near the same environment as possible.

Forkner (1929) suggests the following reasons for the discrepancies in the leucocyte counts:

1. insufficient numbers of animals used.

2. failure to make sufficient number of observations on single animals.

3. lack of adequate methods of counting.

4. confusion of thrombocytes with other elements of the blood.

Seager (1933) believes that platelets account for a large percentage of errors in the count as they average 45,000 per cu mm.

Kyes (1929) states that the hemolysis of the erythrocytes by some stains caused them to resemble white blood cells. These hemolysed cells may have been included in some of the counts, thus giving them a range of approximately twice that of mammals instead of almost the same as recorded by him.

Before 1928, the indirect method of counting was used. This involved the use of a counting chamber and a dry film slide

of stained blood. The number of leucocytes were calculated by setting up a ratio between the total erythrocytes and the total of all cells on the two slides. This method was used by Schmeisser (1915) and gave a range of 20,000 to 80,000 white blood cells per cu mm.

Blain (1928), making a study of tuberculosis in animals, found no satisfactory method of staining the blood of the chicken. He devised the first successful stain for purposes of making the leucocyte count in chickens. His method has been used by various investigators since then, but has not proven entirely satisfactory.

The sex and breed of chickens have some influence on the total count.

Scarborough (1931) in his review of literature states that the male count is higher than that of the female.

Fenstermacher (1932) divided the chickens by sexes and found the range for males at 15,000 to 30,000 and females 19,812 to 29,292 with an average of 24,425. He found a higher count in the White Minorcas chicken than that of the White Leghorn. The Plymouth Rock cockerels having a smaller count than the pullets of the above named breeds.

Cook (1934) found an average difference of 4,000 in favor of the male.

Twisselman (1937) found no differences in the count due to the age of the chickens.

The average counts for chickens found in the reports range

Table 1. Average leucocyte counts of chickens as given by various authors.

Authors	Date	Range	Average	Sex
Bayon, H. P.	1931	16,000-22,500		
Biely, J.	1935	18,300-49,000		F.
Blackmore, F.	1934	17,750-33,300	25,100	
Morning count		13,300-16,000	14,500	
Afternoon		13,000-18,200	15,200	
Blain, D.	1928	10,600-29,460		
Burnett, S. H.	1917		17,921	
Campbell, C. J.	1945	23,100-40,000	28,157	
Cook, F. W.	1934	3,000-47,000	11,000	F.
		9,300-19,100	15,000	M.
Cook, S. F.	1937	30,038-30,980	30,000	
Dougherty, T. F.	1943		11,000	
Emmel, M. W.	1935	29,500-36,000	32,300	
Penstermacher, R.	1932	19,812-29,292	24,425	F.
		15,000-30,000		M.
Forkner, C. E.	1929	6,760-73,600	24,586	
Goodall, A.	1910		19,000	
Kyes, P.	1929	8,000-13,000		
Kydryashov, M. V.	1948		40,000	
Moore, V. A.	1895-6	11,636-28,222	20,281*	
Olson, C. Jr.	1935		47,100	
Schmeisser, H. C.	1915	20,000-80,000	23,675*	
Seager, E. A.	1933		27,000	
Taylor, W. J.	1916	17,300-27,600		
Twisselman, N.	1937	14,500-59,500	38,000	
Ward, A. R.	1904	24,000-61,000	36,185*	
Warthin, A. S.	1907	12,000-29,000		

*Computed from data given in report.

from 11,000, Cook (1934) and Dougherty (1943) to 40,000 reported by Kydryashov (1948).

The different leucocyte counts for chickens found by the various authors is given in Table 1.

MATERIAL AND METHODS

No definite average for the leucocyte count was found anywhere in the literature, nor was there any report of the influence of sex hormones on the leucocyte count located. This study was made to determine if sex hormones influence the leucocyte count in female, male and capon fowls. Numerous blood counts were made on normal, untreated fowls which are believed to give a reliable index to normal levels for purposes of comparison.

This experiment extended throughout the academic year of 1949-1950.

Four separate groups of chickens were used in performing this work, groups I and II were pullets, group III was capons, and group IV cockerels.

Group I consisted of 16 young White Leghorn pullets. These were divided into two pens of eight pullets each on the basis of weight.

Group II was made up of two groups of young pullets obtained from the Kansas State College Poultry Farm. These pullets were divided into groups of five each, according to weight and average leucocyte count which had been determined before

the experiment began.

The pullets in the above groups were approximately the same age. They were kept in separate cages to facilitate feeding and handling. All received the same diet, with food and water available at all times.

The experimental groups were injected intramuscular in the breast region with 0.1 cc of testosterone propionate per injection. The injections were given on Tuesday, Thursday, and Saturday for a period of five weeks. Blood counts were made on Saturdays for a period of seven weeks.

The counts for group II were made on Saturday afternoons from one to three-thirty o'clock, with the controls and then the experimental birds being counted first on alternate weeks. This was done to minimize the effect of a diurnal cycle on the results.

Group III consisted of six White Leghorn cockerels that had been caponized when they were approximately four weeks old. The counts were started when they were three months of age. An average count for this group was obtained before the injections were started. No controls were used, as it was thought that the count established before the injection period could serve as the mean average for the capons.

Injections were given twice weekly for a period of six weeks. Each bird received 0.4 cc of testosterone propionate per week, or a total of 2.4 cc for the entire period.

Ten White Leghorn cockerels approximately eight weeks of

age made up the fourth group. They were divided into two groups of five each on basis of weight and blood count. The experimental group received injections of diethylstilbestrol twice weekly for a period of five weeks. Each injection consisted of 0.3 mg of stilbestrol dissolved in enough corn oil to make 0.1 cc. A total of 0.6 mg per week was given to each bird. The counting was continued over a period of seven weeks, with all counts being made at approximately the same time each week.

The weights of the birds were checked periodically to see that they were progressing normally. If any of the birds showed any visible physical injury, or did not appear healthy during the time the study was being made, they were discarded and not included in the final results.

At the time the count was made the legs and wings were tied and the birds were placed on an operating table, with the right side up, this position exposed the wing vein to be punctured.

Blood was withdrawn from the lateral vein of the wing. The feathers were plucked from this area and the wing was washed before the vein was punctured with a sharp pointed instrument. The blood was allowed to flow freely. A standard white blood cell pipette was used. The blood was drawn up to the 0.5 mark and the pipette was then filled with stain. The stain used was composed of the following substances:

Sodium chloride	3.5 grams
Dextrose	2.5 "
Sodium bicarbonate	0.5 "

Sodium sulfate	2.4 grams
Formalin (conc.)	7.5 ml
Methyl violet (2B)	0.1 gram

The above was diluted with distilled water to make a total volume of 1,000 ml.

The blood of chickens clots very rapidly so it was necessary to obtain the blood as soon as possible after it started flowing from the puncture.

The pipette was shaken from one to three minutes to make sure that the blood was thoroughly mixed and the cells had been stained. The first two drops from the pipette were discarded, and the next two drops were placed on the counting chamber. This mixture was placed under the microscope and the count was made after the cells had settled. The nucleus of the leucocytes stained a deep violet, but the platelets and red blood cells were stained a light violet.

Two groups of 16 squares were counted and the number of leucocytes per cu mm determined from this number. The formula used was as follows: Total number of cells counted divided by the number of squares, times two, times four thousand. An example of this method is: total of 64 cells in 32 squares, equals 2 x 2 x 4,000 or a total of 16,000 white blood cells per cu mm.

No attempt was made toward a differential count of the cells.

RESULTS

The results obtained from the first two groups of chickens were so similar that they will be given together.

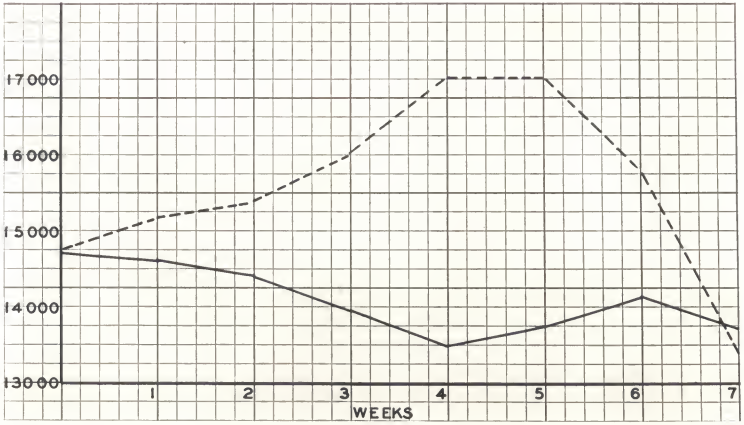
During the five week period in which the injections were administered, the experimental groups showed a steady though irregular weekly increase. The highest counts being registered during the fourth and fifth weeks. Group I reached its peak during the fourth week and remained steady for the fifth week, whereas group II did not reach its peak until the end of the fifth week. During the same period the controls showed an irregular increase and decrease in both groups. The experimental pullets showed a sharp decline during the sixth week, or first week following the last injection. They had not regained their normal level at the end of the seventh week when the counting was discontinued. Plates I and II show the average weekly count over the seven week period of the experiment. The total average white blood cell count for groups I and II were not the same. This difference can be partially explained by their slight differences in age, and the breed of pullets used, as was noted by Fenstermacher (1932). The data given in the plates were computed as the average of all of the chickens in each group. It was treated in this manner as the individual counts per bird fluctuated too greatly to prove significant.

The average leucocyte count for the controls of group I during the seven week period was 14,046 leucocytes per cu mm.

EXPLANATION OF PLATE I

Graph showing the average leucocyte count of six control and six experimental White Leghorn pullets over a seven week period. Experimental birds received injections of testosterone propionate during the first five week period. Solid line represents control and broken line the injected birds.

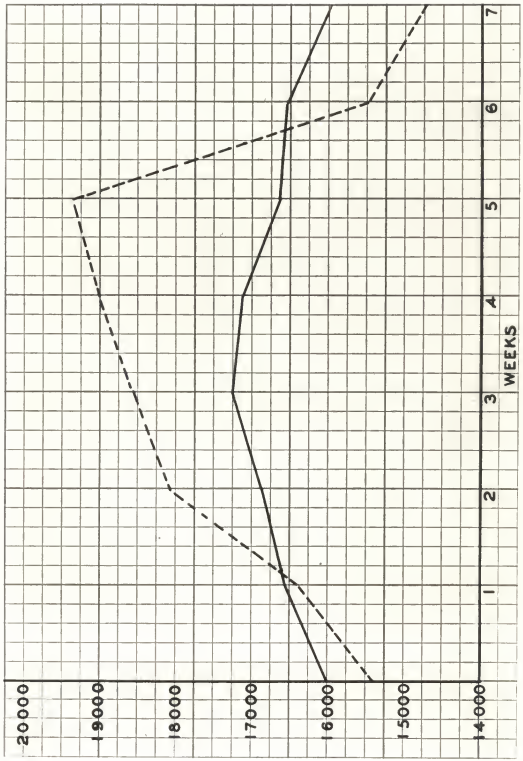
PLATE I



EXPLANATION OF PLATE II

Graph showing the average leucocyte count of five control and five experimental female birds obtained from the Kansas State Poultry Farm. The injected birds received testosterone propionate for the first five weeks. The controls are represented by the solid line and the injected birds with the broken line.

PLATE II



The experimental birds average was 16,130, during the five weeks in which they received the male sex hormone. The highest count of 17,000 was reached during the fourth and fifth weeks. Group II had a higher initial count, their average being 16,705 for the controls. The experimental birds maintained an average of 18,736 over the five week injection period. A high of 19,440 was reached on the final week of the injection period.

The average differences between the controls and the experimental birds of these two groups show a close correlation. In group I the difference was 2,084, and in group II it was 2,031 white blood cells per cu mm.

To determine the leucocyte count for group III a series of counts were made prior to the period of injection. The average count for the six birds during this period was used as the normal count and no controls were used. These birds showed a steady increase during the six week injection period. When the injections were stopped the decline was rapid. The count was still on the decline at the end of the eighth week when counting was discontinued.

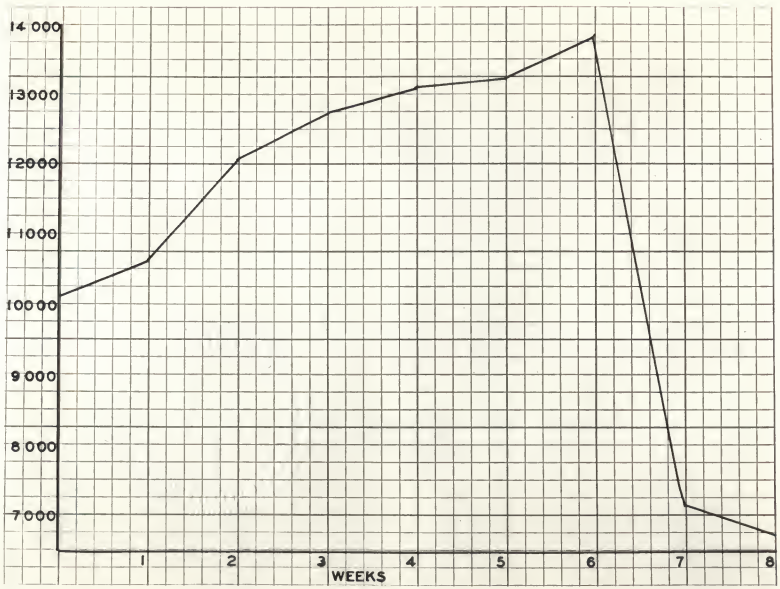
The count established for the normal capons was much lower than had been expected. The total count determined before injection was 10,150. This had increased to 13,890 by the end of the sixth week, but was still lower than the count for the normal White Leghorn cockerels. Plate III shows the increase and decline registered by this group.

A control and an experimental group were used in this

EXPLANATION OF PLATE III

Graph showing the average leucocyte count of six White Leghorn capons during an eight week period. Each bird received injections of 10 mg of testosterone propionate weekly for the first six weeks.

PLATE III


14 000
13000
12000
11000
10000
9000
8000
7000
1
2
3
4
5
6
7
8
WEEKS

series of counts. The data found are given in Plate IV. The average count for this group was found to be 15,720. The count for the birds receiving the injections of stilbestrol declined steadily after the first week. By the third week it had fallen below that found to be normal for the White Leghorn pullet. The leucocyte number rebounded immediately after the last injection, and by the end of the seventh week had almost reached the normal level determined for this group.

CONCLUSION

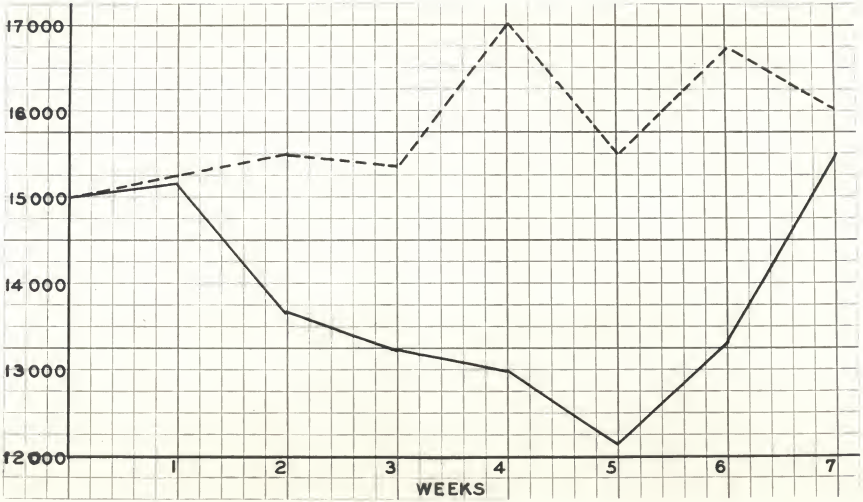
The evidence found in the first two experiments with the pullets seems to indicate that the differences in total leucocyte count in the male and female chicken is due in part to the sex hormones. The male hormone accounting for more white blood cells to be present per cubic millimeter of blood. The injected White Leghorn pullets showed an increase that was practically identical with the count established for the males used in the fourth experiment. No comparison between the injected females in group II and males of their breed can be made from this work as no counts were made on the males of this breed.

The capons, showing a very low count before the injection period, increased with the injections. The count had not reached the normal male level after six weeks of injections, even though the male characters were evident in the comb and wattles. The count did increase almost 3,000 cells due to the

EXPLANATION OF PLATE IV

Graph showing the average leucocyte count for five control and five experimental White Leghorn cockerels. The five injected cockerels received .6 mg of diethylstilbestrol weekly for a period of five weeks during the seven weeks in which the counts were made.

PLATE IV



male sex hormone received.

The cockerels, contrary to the findings of Tyslowitz (1941), and Crafts (1941) in their work with the Rhesus monkey, registered a definite decrease in leucocyte count following the repeated injections of diethylstilbestrol. The count decreased during the injection period to a level slightly below that of the normal White Leghorn pullet.

The data obtained from the four experimental groups show in these cases that testosterone propionate in dosages of 0.5 cc per week in the normal female chicken and in the capon causes an immediate and significant increase in the total number of leucocytes per cu mm, and that diethylstilbestrol in amounts of 0.6 mg per week causes an immediate decrease in the leucocyte count in cockerels.

SUMMARY

1. The normal leucocyte count for the White Leghorn pullet was established at 14,046 per cu mm. Injections of testosterone propionate in dosages of 7.5 mg per week for a period of five weeks caused the leucocytes to increase to an average count of 16,130 per cu mm. This total is almost identical with that found for the White Leghorn cockerels.

2. In group II the crossbreed pullets, obtained from the Kansas State Poultry Farm, showed an average increase of 2,031 leucocytes. Although all counts in this group were higher than

in group I, the increase corresponds very closely.

3. White Leghorn capons registered an increase in number of cells following injections of testosterone, but did not reach the normal White Leghorn cockerel level.

4. The leucocyte count for the White Leghorn cockerels was found to average 15,720. The count decreased below that of the pullet after a five week period of injections, in which 0.6 mg of diethylstilbestrol was administered each week. The count returned to near normal level two weeks following the last injection.

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